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No Evidence for Linkage of Chromosome 22 Markers to Schizophrenia in Southern African Bantu-Speaking Families

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Previous studies have demonstrated possible linkage between chromosome 22 and one of the hypothesized schizophrenia susceptibility genes. Interpretation of these data, however, is not straightforward: although not significant at the level traditionally accepted to demonstrate linkage, reported lod scores were greater than should have occurred by chance for an unlinked marker based on simulation studies. Further, these studies used sample populations which were either of mixed nationality and ethnicity, or mixed ethnic ancestry from one country. We therefore tested for linkage between highly polymorphic chromosome 22 markers and schizophrenia in a sample of southern African Bantu-speaking black families, a population known to have diverged within the last 2,000 years. We also tested one candidate locus, the gene for the soluble form of catechol-O-methyl transferase (COMT) located at 22q11, which has been suggested as the cause of psychiatric symptoms observed in velo-cardio-facial syndrome (VCFS, including DiGeorge syndrome), and which is known to be functionally as well as genetically polymorphic. There is no evidence to support the linkage of markers on chromosome 22 to susceptibility to schizophrenia in this population, using either parametric or nonparametric analysis. © 1996 Wiley-Liss, Inc.

KEY WORDS: South African, linkage analysis, parametric, nonparametric, catechol-O-methyl transferase

INTRODUCTION

A large body of older data demonstrates the presence of familial genetic factors in schizophrenia. Studies of twins, adoptees, and families [reviewed by Gottesman and Shields, 1982; Kendler and Diehl, 1993] show increasing concordance with increasing degree of relationship and a greater role of genetic as compared to environmental effects. Both the nature and magnitude of the results are reproducible, and are robust to reevaluation with newer, more powerful analytical methodology [Kendler et al., 1981; McGuffin et al., 1984; Farmer et al., 1987]. Monozygotic twin concordance approximately three times that for dizygotic twins is compelling evidence for the presence of etiologies which are in large part genetic, at least in familial cases.

Three recent reports from two independent genome scans have suggested that loci at chromosome 22q12–q13.1 are linked to susceptibility to schizophrenia [Pulver et al., 1994a; Coon et al., 1994a,b]. These reports, however, provided only weak evidence for involvement of this region, with lod scores between 1.5–2.0 using large cohorts of nuclear families. These results are not significant at the level of 3.0 traditionally required for demonstrating linkage, but are worth investigating based on the more flexible approach taken to mapping oligogenic and multifactorial diseases [Davies et al., 1994; Hashimoto et al., 1994], especially those where the genetic complement is thought to lead to susceptibility rather than to direct expression of phenotype.

We chose to study a sample of South African Bantu-speaking black families drawn from the population of the townships around Johannesburg. All distinct South African black groups (Zulu, Xhosa, Sotho, Tswana, etc.) are Bantu language speakers. Linguistic data show that, although separate, these languages are such closely related members of a single, large language family that it is unlikely that the time since their divergence can be very great [Nurse et al., 1985]. The migration south of the original population of Bantu-speakers began around the Bight of Benin in modern Nigeria some 2,000–3,000 years ago. Linguistic diver-

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gence, and thus by extension genetic divergence, between distinct modern groups can begin no earlier than this. Further, as South Africa is the southern limit of this predominantly southern migration, it is likely that the divergence of local groups began much later. Because of these historical circumstances which gave rise to local populations, the precise combination of contributing genetic factors in affected or susceptible individuals is much more likely to be the same in a group such as the South African Bantu-speaking blacks than it is in a population of mixed ethnicity and diverse geographical background, such as North Americans. Ramsay and Jenkins [1987] provided genetic evidence that this is the case by showing that at both the northern and southern extremities of Bantu-speaking Africa, 87% of β^s sickle-cell mutations occur on the same background haplotype.

We collected 23 families multiply affected with schizophrenia from the Bantu-speaking population of the catchment area of the Psychiatric Department, Baragwanath Hospital, Soweto. The sample represents cases from all the related linguistic subgroups in the South African black population (Zulu, Xhosa, Sotho, Tswana, etc.). We tested for linkage between schizophrenia and a comprehensive set of highly informative chromosome 22 markers in these families, and we also analyzed catechol-O-methyltransferase (COMT) as a candidate gene for the phenotype.

MATERIALS AND METHODS

Ascertainment and Diagnosis

Patients were initially ascertained by Baragwanath Hospital's usual practice of team consensus diagnosis, which has recently been validated for psychiatric genetic research [Maziade et al., 1992] using DSM-III-R [American Psychiatric Association, 1987] criteria. Only patients diagnosed as schizophrenic were interviewed to determine those with other family members affected, and those families with 2 or more affected members were asked to participate. Patients with a possible diagnosis of alcohol- or substance-abuse-induced psychosis were excluded. Families who agreed were then interviewed member by member using the Structured Clinical Interview for DSM-III-R Patient and Nonpatient (SCID-P and SCID-NP) versions [Spitzer et al., 1990], as appropriate, by one of us (M.M.C.). All diagnoses were made blind to any genotypic information. One family which contained 2 affected monozygotic twins, and no other affected individuals, was dropped from the study. Twenty-two families with 2 or more affected members whose diagnoses were the same in both rounds of ascertainment were selected as the study population (n = 97, including 58 cases of DSM-III-R schizophrenia). Full details of the sample are presented elsewhere [Riley et al., 1996]. Two of these families were missing critical affected samples, and were not included in the analyses presented here. Blood samples were collected by a district nurse. Ascertainment and sample collection are continuing currently, in order to add greater power to subsequent investigations. The preferential selection of loaded pedigrees should not introduce bias with respect to detection of genetic linkage [Ott, 1991].

Genetic Markers

Nine previously described polymorphic loci were chosen in the region 22q11–q13, i.e., eight highly polymorphic short tandem repeats (STRs) [Buetow et al., 1993; Gyapay et al., 1994] and one restriction fragment length polymorphism (RFLP) [Tenhunen et al., 1994]. Four of the STRs have heterozygosities >0.8, three >0.6, and one >0.5. The single RFLP, which is a functional polymorphism in the catechol-O-methyltransferase (COMT) gene, was investigated as a potential candidate, and has a heterozygosity of only 0.3 in this population. The marker map is *F8VWFP*–16 cM–*COMT*–1.5 cM–*D22S264*–7.8 cM–*D22S303*–1.9 cM–*D22S257*–16 cM–*D22S421*–11 cM–*D22S2815* cM–*D22S277*–2 cM–*D22S283*. The total genetic distance between the most centromeric and most telomeric markers is 61.2 cM (or 83.2% of the total genetic length of chromosome 22) based on the most recent Genethon [Gyapay et al., 1994] and Collaborative Human Linkage Center (CHLC) integrated maps [Murray et al., 1994]. They have an average intermarker distance of 7.35 cM.

Genotyping

High molecular weight DNA was prepared from fresh whole blood or frozen buffy coats by standard methods [Miller et al., 1988]. To visualize STRs, 20 ng of genomic DNA were amplified by polymerase chain reaction (PCR), using 6.25 pmol of each primer in 12.5 μl of standard PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatine, 200 μM dCTP, dGTP, dTTP, 25 μM dATP (Pharmacia, St. Albans, UK), and 0.5 U Taq polymerase (Ampli-Taq, Cetus, Warrington, UK), with the addition of 0.625 μCi [α^{33}P]dATP (Amersham, Aylesbury, UK) for 35 cycles at primer-specific annealing temperatures. Where the annealing temperatures allowed, PCR reactions were duplexed: 12.5 μl of loading buffer (98% (v/v) formamide, 10 mM EDTA, 0.025% (w/v) xylene cyanol, and 0.025% (w/v) bromophenol blue) were added to each reaction tube, and 4 μl of this mix were loaded onto denaturing 6% polyacrylamide/7 M urea/1 × TBE gels (stock solution from Severn Biotech, Kidderminster, UK), and eletrophoresed at 60 W constant power for 1.5–2.5 hr, depending on product size in 1 × TBE (90 mM Tris-borate, 2 mM EDTA). Dried gels were exposed to Biomax MR autoradiography film (Kodak, Cambridge, UK) for 16 hr–7 days depending on signal strength.

From sequence data on the COMT gene [Tenhunen et al., 1994], two primers, COMT-F: 5′-ACTGTGGCTACTCAGCTGTG-3′ (annealing temperature, 62°C) and COMT-R: 5′-CCTTTTTCCAGGTCTGACAA-3′ (annealing temperature, 58°C), were designed to amplify across the RFLP site, yielding a product of 169 base pairs (bp). Thirty ng of genomic DNA were amplified with 12.5 pmol of each primer in 25 μl of standard buffer (as above, except for 200 μM each dNTP, and 1 U Taq polymerase) for 30 cycles using an annealing temperature of 60°C. Fifteen μl of the reaction were mixed with 10 units of *Nla*III (New England Biolabs, Hitchin,

UK), 2 μl of the manufacturer's supplied buffer, and 100 μg/ml acetylated bovine serum albumin, brought up to 20 μl with sterile H_2O, and digested for 1 hr at 37°C. Digests were then electrophoresed on nondenaturing 6% polyacrylamide/1 × TBE minigels (stock solution from Severn Biotech) in 1 × TBE, stained with ethidium bromide, destained, and photographed. A band at 169 bp indicated no *Nla*III restriction site in the PCR product, and higher-activity COMT. A band at 129 bp indicated the presence of the *Nla*III site (the 40-bp fragment was not visible under these conditions), and a change from G to A at position 544 of the COMT gene sequence, producing a Val→Met change in the amino acid sequence, and the lower activity enzyme (Fig. 1).

Lod Score Analyses

Two-point linkage analyses between schizophrenia and the chromosome 22 markers were performed using the FASTLINK [Cottingham et al., 1993; Schaffer et al., 1994] version of MLINK from the LINKAGE package [Lathrop and Lalouel, 1984]. Models used for parametric analysis are presented in Table I. We have varied penetrance values from 0.25–0.85 for the heterozygote using a model of codominant inheritance, which given the values of q obtained is equivalent to a dominant

TABLE I. Analysis Model Parameters

Codominant, penetrance	*f*1	*f*2	*f*3	q
0.25	0.001	0.25	0.5	0.0153
0.5	0.001	0.5	1.0	0.0076
0.6	0.001	0.6	1.0	0.0064
0.85	0.001	0.85	1.0	0.0045
Recessive, penetrance				
0.5	0.001	0.001	0.5	0.1234
0.6	0.001	0.001	0.6	0.1126

analysis, since the frequency of homozygotes under any of the models tested would be expected to be <1 in a sample of this size. Under a recessive model of inheritance, we tested penetrance values of 0.5 and 0.6. We also constructed 12 age- and gender-dependent liability classes based on age of onset data from another African population [Gureje, 1991], as shown in Table II. We assume a phenocopy risk, *f*1, of 0.001. Only a narrow diagnostic model is used in this sample, corresponding to DSM-III-R schizophrenia. No other diagnoses have been found in these families. Although this sample group was chosen to minimize the potential for hetero-

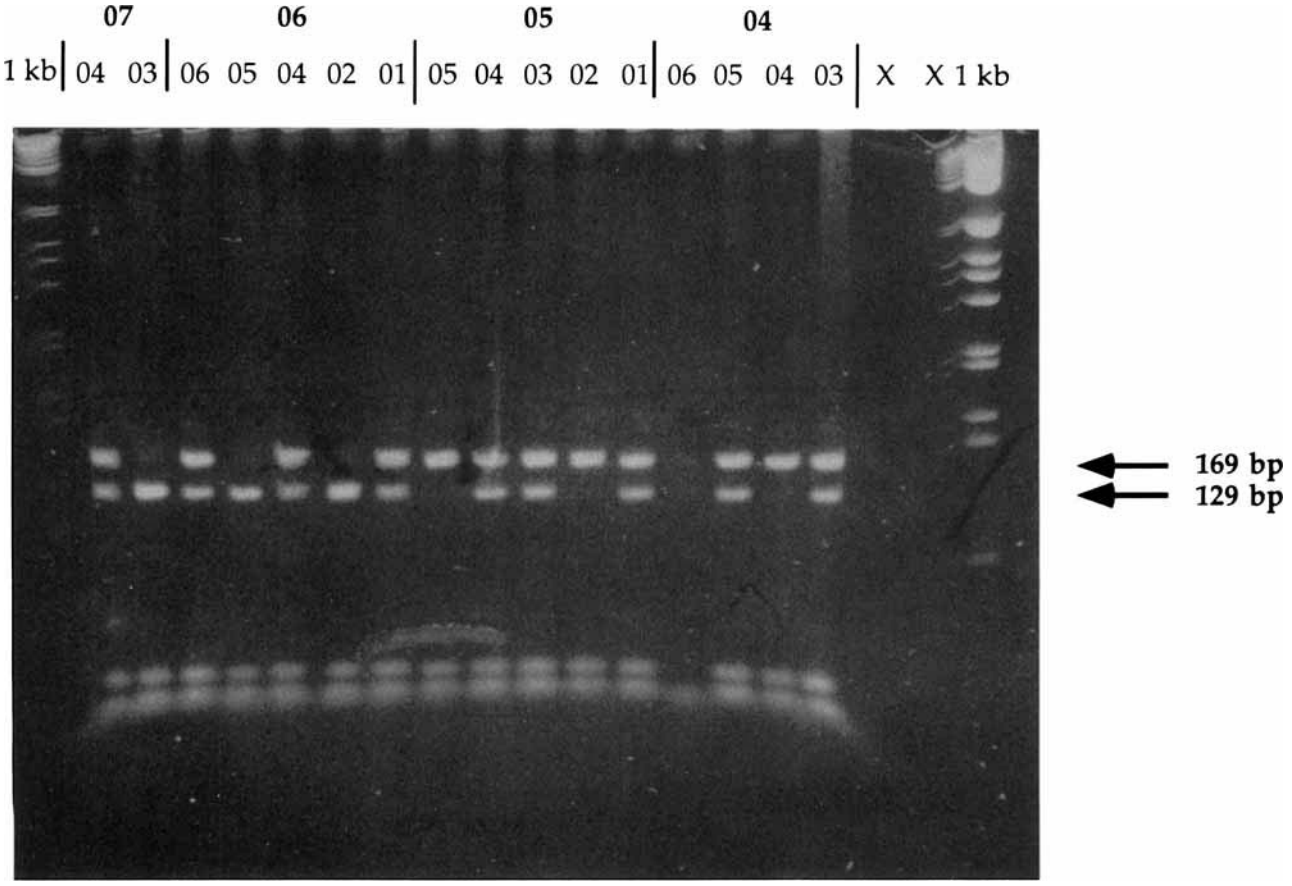

Fig. 1 COMT polymorphism, in members of families 04–07. Mendelian inheritance is observed for this polymorphism, and can be seen in families 05 and 06.

TABLE II. Age- and Gender-Dependent Liability Classes Based on Cumulative Percentages of Patients With First Episode by Start of Age Interval*

Liability class	Age	Gender	Cumulative %
1	40+	M	98.4
2		F	97.8
3	35–39	M	94.4
4		F	83.1
5	30–34	M	83.2
6		F	66.3
7	25–29	M	64.8
8		F	44.9
9	20–25	M	24.8
10		F	16.9
11	16–19	M	4.0
12		F	1.1

* Data from Gureje [1991].

geneity, we tested for homogeneity using the HOMOG program [Ott, 1991].

Affected Pedigree Member Analysis

Deviations from the allele-sharing frequencies expected by chance between family members are also evidence for linkage, and do not require the estimation of parameters, such as penetrance, the true values of which are unknown. Because of the structure of our families, sib-pair analysis does not use all the information present in our sample. In order to be certain that misspecification of model parameters has not led to false exclusion results, we analyzed the data using the affected pedigree-member analysis program APM [Weeks and Lange, 1988] to test for excess sharing of alleles.

RESULTS

Two-Point Analysis

Tables III and IV present the results of two-point linkage analyses on data from the nine chromosome 22 markers. The COMT polymorphism was not excluded under any of the codominant models tested, but was excluded under a recessive model with penetrance of 0.6, and gave a lod score of −1.814 under a recessive model with penetrance of 0.5 (corresponding to 65 times greater likelihood of the marker being unlinked to the phenotype, given these results, than linked to it).

Marker F8VWFP was not excluded at $\Theta = 0.00$ under either of the recessive models tested. Under a codominant model with penetrance of 0.25, it was excluded only at $\Theta = 0.00$. Under the same model, the two most telomeric markers, D22S277 and D22S283, were also not formally excluded at $\Theta = 0.00$, but yielded lod scores of −1.817 and −1.667, respectively (corresponding to 66 and 46 times greater likelihoods, respectively, of the markers being unlinked to the phenotype, given these results, than linked to it). With the exception of these specific results, all other marker/model combinations yielded formal exclusion intervals between 2–16 cM. Figure 2 shows the exclusion intervals obtained for each marker under each model tested.

Under codominant models, the highest lod score was 0.296 for marker D22S283 at $\Theta = 0.23$ (penetrance of 0.85). This is much less than the estimated maximum lod score for an unlinked marker based on simulation data, and a lod score >0 would be expected at this value of Θ for an unlinked marker about 24% (95% confidence interval: ±8.6%) of the time. Under recessive models, the highest lod score was 0.364 for marker F8VWFP at $\Theta = 0.18$ (penetrance of 0.6). Simulated pedigrees show that a lod score >0 would be expected at this value of Θ for an unlinked marker 32% (95% confidence interval: ±9.6%) of the time. Although all available evidence suggests that this population will not show significant within-group heterogeneity, we tested for the presence of such an effect using the results from marker D22S257, because of the results obtained from nonparametric analyses (see below). Results of homogeneity analysis are presented in Table V. As expected, there is no evidence whatsoever of heterogeneity in this sample group.

Affected Pedigree Member Analysis

Results of these analyses, including "empirical" *P*-values based on simulated replicates of 1,000 families, are presented in Table VI. Under the two more conservative weighting functions ($f(p) = 1$, $f(p) = 1/\sqrt{p}$), there was no evidence for increased allele sharing in the affected individuals. Under the least conservative weighting function ($f(p) = 1/p$), there was evidence for increased sharing of alleles at both the COMT locus and marker D22S257. In the case of the COMT locus, the sample *P* value was 0.041, and the empirical *P* value, 0.064. This is likely due to the low frequency of the low-activity allele, and excess sharing of the high-activity allele, which in turn is unlikely to be etiologically relevant. The COMT polymorphism may be involved in liability to psychotic illness through haploinsufficiency of this rate-limiting enzyme either in a dominant manner (if less than two copies of the normal activity gene increases liability) or a recessive manner (if less than one copy of the normal activity gene increases liability). Either of these situations would require excess sharing of at least one copy of the low-activity allele, which was not observed. The initial suggestion of possible COMT involvement in psychotic illness was that individuals hemizygous at this locus with a low-activity allele on the nondeleted chromosome might have increased liability to psychosis [Dunham et al., 1992], but there has so far been no evidence to support this. In the case of marker D22S257, the sample *P* value was <0.00001, and the empirical *P* value was 0.003. However, the bulk of this effect was due to a single family (pedigree 21) with fully informative parents sharing a single rare allele (frequency = 0.025), and with both affected children homozygous for that allele. Given that these results only achieve significance under the least conservative weighting function and that the effect is clustered in a single pair of affected siblings, in combination with the strongly negative LINKAGE results for this locus, it is unlikely that they represent a true positive finding, although allelic association

with the disease, especially in pedigree 21, cannot be ruled out.

DISCUSSION

Assuming either codominant or recessive inheritance, we find no evidence for linkage of schizophrenia to markers on the long arm of chromosome 22 using a number of different penetrance values. Several lod scores >0 are found, for markers F8VWFP and D22S283, but only at large values of θ. The magnitudes of both these positive lod scores are well within the range expected by chance for unlinked markers at the values of θ where they occur. Furthermore, the values of θ for which these results occur suggest locations either 1) within the exclusion intervals of other markers in the current study, or 2) on the short arm in the case of F8VWFP and at or beyond the telomere in the case of D22S283. Similarly, nonparametric affected pedigree-

TABLE III. Results of Two-Point Analyses Under Codominant Models

	Lod scores, penetrance of 0.25						
				θ			
Marker	0.00	0.01	0.02	0.03	0.05	0.1	0.15
F8VWFP	−2.107	−1.822	−1.599	−1.416	−1.132	−0.675	−0.406
COMT	−0.645	−0.604	−0.566	−0.530	−0.464	−0.329	
D22S264	−2.858	−2.339	−1.964	−1.675	−1.249	−0.635	
D22S303	−3.684	−3.121	−2.703	−2.372	−1.872	−1.101	
D22S257	−3.534	−2.969	−2.549	−2.218	−1.718	−0.960	−0.542
D22S421	−4.034	−3.308	−2.821	−2.454	−1.920	−1.133	−0.696
D22S281	−2.719	−2.406	−2.158	−1.952	−1.621	−1.058	
D22S277	−1.817	−1.511	−1.275	−1.085	−0.798	−0.373	
D22S283	−1.666	−1.291	−1.020	−0.813	−0.514	−0.111	
	Lod scores, penetrance of 0.5						
				θ			
Marker	0.00	0.01	0.02	0.03	0.05	0.1	0.15
F8VWFP	−3.204	−2.501	−2.085	−1.787	−1.367	−0.761	−0.429
COMT	−0.802	−0.750	−0.701	−0.656	−0.573	−0.404	
D22S264	−4.181	−3.412	−2.681	−2.194	−1.551	−0.721	
D22S303	−5.508	−4.264	−3.541	−3.029	−2.316	−1.303	
D22S257	−5.471	−4.029	−3.251	−2.719	−1.999	−1.025	−0.531
D22S41	−5.793	−4.175	−3.381	−2.852	−2.147	−1.197	−0.704
D22S281	−3.480	−2.833	−2.432	−2.136	−1.703	−1.041	
D22S277	−3.323	−2.489	−1.994	−1.644	−1.164	−0.516	
D22S283	−2.842	−1.993	−1.503	−1.164	−0.709	−0.136	
	Lod scores, penetrance of 0.6						
				θ			
Marker	0.00	0.01	0.02	0.03	0.05	0.1	0.15
F8VWFP	−3.559	−2.655	−2.182	−1.856	−1.405	−0.765	−0.419
COMT	−0.857	−0.802	−0.750	−0.701	−0.611	−0.431	
D22S264	−5.487	−3.655	−2.818	−2.280	−1.589	−0.713	
D22S303	−6.128	−4.598	−3.782	−3.219	−2.448	−1.363	
D22S257	−6.167	−4.277	−3.388	−2.803	−2.033	−1.013	−0.505
D22S421	−6.260	−4.333	−3.464	−2.901	−2.163	−1.187	−0.687
D22S281	−3.674	−2.885	−2.441	−2.123	−1.669	−0.993	
D22S277	−3.921	−2.802	−2.210	−1.807	−1.264	−0.544	
D22S283	−3.280	−2.222	−1.657	−1.275	−0.770	−0.137	
	Lod scores, penetrance of 0.85						
				θ			
Marker	0.00	0.01	0.02	0.03	0.05	0.1	0.15
F8VWFP	−4.249	−2.871	−2.312	−1.942	−1.442	−0.741	−0.364
COMT	−1.132	−1.056	−0.985	−0.918	−0.798	−0.557	
D22S264	−7.296	−4.239	−3.202	−2.568	−1.766	−0.753	
D22S303	−7.771	−5.505	−4.489	−3.810	−2.887	−1.587	
D22S257	−7.940	−4.802	−3.718	−3.043	−2.174	−1.038	−0.475
D22S421	−7.178	−4.553	−3.570	−2.956	−2.169	−1.147	−0.634
D22S281	−4.096	−2.978	−2.454	−2.092	−1.586	−0.858	
D22S277	−5.721	−3.749	−2.899	−2.339	−1.597	−0.637	
D22S283	−4.818	−3.095	−2.299	−1.770	−1.076	−0.210	
D22S281	−5.609	−4.765	−4.134	−3.632	−2.866	−1.681	
D22S277	−4.080	−3.101	−2.444	−1.955	−1.261	−0.326	
D22S283	−5.207	−4.234	−3.546	−3.010	−2.207	−1.021	

TABLE IV. Results of Two-Point Analyses Under Recessive Models

	Lod scores, penetrance of 0.5						
				θ			
Marker	0.00	0.01	0.02	0.03	0.05	0.1	0.15
F8VWFP	−0.881	−0.643	−0.462	−0.318	−0.104	−0.187	−0.295
COMT	−1.814	−1.525	−1.304	−1.128	−0.860	−0.456	
D22S264	−3.365	−2.756	−2.291	−1.919	−1.354	−0.520	
D22S303	−4.796	−4.118	−3.584	−3.143	−2.450	−1.346	
D22S257	−4.871	−4.061	−3.451	−2.965	−2.230	−1.123	−0.535
D22S421	−4.734	−4.117	−3.617	−3.199	−2.535	−1.467	−0.851
D22S281	−4.933	−4.277	−3.759	−3.333	−2.666	−1.599	
D22S277	−3.322	−2.619	−2.104	−1.703	−1.114	−0.290	
D22S283	−4.411	−3.686	−3.130	−2.680	−1.986	−0.928	
	Lod scores, penetrance of 0.6						
				θ			
Marker	0.00	0.01	0.02	0.03	0.05	0.1	0.15
F8VWFP	−1.081	−0.767	−0.542	−0.368	−0.114	−0.222	−0.346
COMT	−2.165	−1.781	−1.506	−1.293	−0.978	−0.515	
D22S264	−4.119	−3.276	−2.678	−2.217	−1.539	−0.569	
D22S303	−5.545	−4.653	−3.995	−3.470	−2.665	−1.424	
D22S257	−5.798	−4.675	−3.896	−3.302	−2.435	−1.181	−0.536
D22S421	−5.322	−4.540	−3.940	−3.453	−2.696	−1.514	−0.852
D22S281	−5.609	−4.765	−4.134	−3.632	−2.866	−1.681	
D22S277	−4.080	−3.101	−2.444	−1.955	−1.261	−0.326	
D22S283	−5.207	−4.234	−3.546	−3.010	−2.207	−1.021	

member analysis gave no evidence for increased allele sharing under the two more conservative weighting functions, and although two P values <0.05 were generated by the least conservative weighting function, these results are equivocal, and must be interpreted cautiously and in light of the sample data. Importantly, there is no evidence of heterogeneity in this sample group, supporting our hypothesis that the South African Bantu-speaking black population is an excellent resource for molecular genetic analysis of complex diseases.

Psychiatric symptoms (psychosis, affective disorder, and learning or behavioral difficulties) commonly present among the syndromes known alternatively as velocardio-facial syndrome (VCFS) or DiGeorge syndrome (DGS) [Shprintzen et al., 1992], which is associated with interstitial deletions of chromosome 22q11 [Scambler et al., 1992; Kelly et al., 1993]. VCFS is a

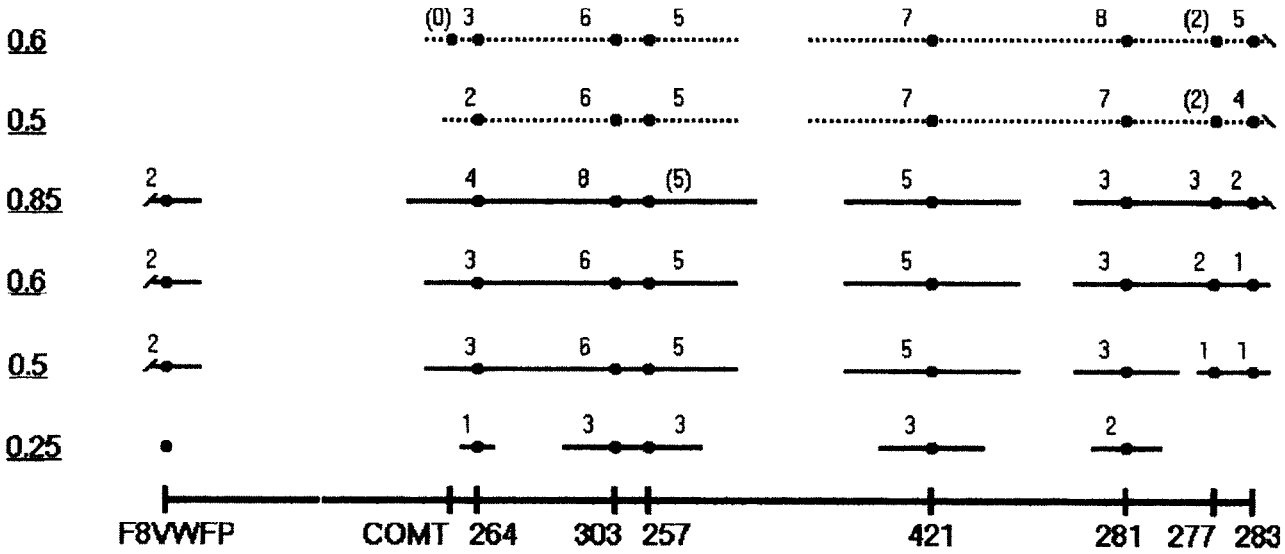

Fig. 2 Exclusion intervals obtained for chromosome 22 markers. Penetrance values at which the corresponding results were obtained are shown at left. Solid lines represent codominant models, and dashed lines recessive models. Numbers above interval bars are centimorgans (cM) formally excluded ($Z < -2$) on either side of the marker. Where a marker symbol is present without interval bars, it was formally excluded on at $\Theta = 0.00$.

TABLE V. Results of Heterogeneity Analysis From Lod Scores for Marker D22S257 Under a Codominant Model With Penetrance of 0.05

Hypotheses	Max. In L	Alpha	Theta
H2: linkage, heterogeneity	0.0001	0.0500	0.1000
H1: linkage, homogeneity	0.0000	(1)	99.0000
H0: no linkage	(0)	(0)	(0.5)
Components of chi-square	**df**	**Chi-square**	**L ratio**
Source			
H2 vs. H1 heterogeneity	1	10.000	1.0001
H2 vs. H0 linkage	1	0.000	1.0000

multiple congenital anomaly disorder of development, and the critical deletion region is therefore very likely to contain genes which are important in the neurodevelopmental process. It is also known to contain the gene for catechol-O-methyl transferase (COMT) [Dunham et al., 1992], an important enzyme in the synthetic and degradatory pathways of catecholamines. Its soluble form (S-COMT) has high- and low-activity alleles [Weinshilboum and Raymond, 1977], and the single base change which causes the differential activity is detectable as a restriction fragment length polymorphism with *Nla*III [Tenhunen et al., 1994]. Disruptions of catecholamine metabolism, especially of the dopaminergic system, are some of the most robust results from the long history of investigation into the biology of schizophrenia. Although the functional polymorphism in the COMT gene may be responsible for the psychiatric symptoms seen in VCFS, due to the presence of a low-activity allele in an individual hemizygous for this region, we find no evidence for a role for this gene in the etiology of schizophrenia in this population. This is in agreement with another recent report of lack of association between schizophrenia and alleles at the COMT locus using our PCR assay [Daniels et al., 1996].

These results are in general agreement with those found by the collaborative groups attempting to replicate the findings of Pulver et al. by parametric [Polymeropoulos et al., 1994; Pulver et al., 1994b; Vallada et al., 1995; Kalsi et al., 1995] and nonparametric [Gill et al., 1996] analysis. Although the last report does find evidence for a susceptibility locus for schizophrenia near D22S278, the authors conclude that it is likely to be responsible for no more than 2% of the variance in liability to develop schizophrenia. Our results for marker D22S277 (which is only 1 cM away from D22S278) are negative in both parametric and nonparametric analyses, indicating little chance of such an effect in this group.

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TABLE VI. Results of Affected Pedigree Member Analysis

			Allele frequency weighting function					
			$f(p) = 1$		$f(p) = 1/\sqrt{P}$		$f(p) = 1/P$	
Locus	Families[a]	Affecteds[b]	Sample P	Empirical P	Sample P	Empirical P	Sample P	Empirical P
FV8WFP	15	35	0.33638	0.327	0.32852	0.311	0.58861	0.470
COMT	12	29	0.52312	0.522	0.16069	0.169	0.04102*	0.064
D22S264	15	35	0.70657	0.700	0.88254	0.904	0.73435	0.948
D22S303	15	35	0.70858	0.687	0.83642	0.858	0.64438	0.884
D22S257	15	35	0.98012	0.988	0.11270	0.108	0.00000**	0.003**
D22S421	12	29	0.64807	0.654	0.42301	0.389	0.40843	0.320
D22S281	14	32	0.67911	0.692	0.53866	0.550	0.49017	0.435
D22S277	15	35	0.69640	0.676	0.68432	0.690	0.65021	0.655
D22S283	15	35	0.63460	0.634	0.64472	0.607	0.56720	0.489

[a] Number of families used in APM calculations (families with only a parent-child pair are excluded).
[b] Total number of affected individuals used in calculations.
* $P < 0.05$.
** $P < 0.005$.

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